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L1: Entry 4 of 4 File: USPT Apr 11, 2000

US-PAT-NO: 6048715
DOCUMENT-IDENTIFIER: US 6048715 A

TITLE: Two-phase partition affinity separation system and affinity separated cell-containing composition

DATE-ISSUED: April 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/179; 435/178, 435/320.1, 435/395, 435/68.1, 435/69.1, 435/69.7, 435/70.1, 435/71.1, 435/71.2, 435/803 , 435/815, 436/529, 436/530, 530/413, 530/813, 530/814

CLAIMS:

What is claimed is:

1. A two-phase partition system for affinity separation comprising:

as a first component a phase-forming oligosaccharide polymer to which a polysaccharide binding peptide binds with a Ka of 10.sup.3 M to 10.sup.7 M and as a second component a phase separation inducing agent selected from the group consisting of a polyethylene glycol polymer, a dextran, a copolymer of ethylene oxide and propylene oxide, and a salt at a concentration of at least 3 M, wherein said first and second components are each present in an amount sufficient to induce phase separation upon mixing of said first component and said second component, wherein said first component is selected from the group consisting of methyl cellulose, and ethylhydrokyethyl cellulose.

2. A composition comprising:

a polypeptide which comprises a non-catalytic polysaccharide binding peptide bound to (1) a phase-forming oligosaccharide polymer and (2) a cell having a carbohydrate residue on its surface to which said polysaccharide binding peptide binds, wherein said composition is obtained by the method of contacting said polysaccharide binding peptide with said cell to form a complex with said cell and contacting said complex with a two-phase partition system which comprises as a first phase a phase-forming oligosaccharide polymer to which said polysaccharide binding peptide binds with a Ka of 10.sup.3 M to 10.sup.7 M, and as a second phase a phase separation inducing agent selected from the group consisting of a polyethylene glycol polymer, a dextran, and a copolymer of ethylene oxide and propylene oxide, and a salt at a concentration of at least 3 M, whereby said complex partitions into said first phase by binding to said phase-forming oligosaccharide polymer; and recovering said first phase, whereby said composition is obtained.

3. The composition according to claim 2, wherein said phase-forming oligosaccharide

polymer is a .beta.-1,4-glucan.

4. The composition according to claim 3, wherein said .beta.-1,4-glucan is a cellulose.

5. The composition according to claim 3, wherein said .beta.-glucan is obtainable from a cereal.

6. The composition according to claim 5, wherein said cereal is oat or barley.

7. The composition according to claim 2, wherein said polysaccharide binding peptide is derived from a polysaccharide binding domain of a polysaccharidase.

8. The composition according to claim 7, wherein said polysaccharidase is a cellulase.

9. A composition comprising:

a polypeptide which comprises a non-catalytic polysaccharide binding peptide bound to (1) a phase-forming oligosaccharide polymer and (2) a cell having a carbohydrate residue on its surface to which said polysaccharide binding peptide binds, wherein said composition is obtained by the method of contacting said polysaccharide binding peptide with said cell to form a complex with said cell and contacting said complex with a two-phase partition system which comprises as a first phase a phase-forming oligosaccharide polymer selected from the group consisting of hydroxyethyl cellulose, carboxymethyl cellulose, methyl cellulose, ethylhydroxyethyl cellulose and hydroxypropyl cellulose, and as a second phase a phase separation inducing agent selected from the group consisting of a polyethylene glycol polymer, a dextran, and a copolymer of ethylene oxide and propylene oxide, and a salt at a concentration of at least 3 M, whereby said complex partitions into said first phase by binding to said phase-forming oligosaccharide polymer; and recovering said first phase, whereby said composition is obtained.

10. The composition according to claim 9, wherein said polysaccharide binding peptide is derived from C. fimi endoglucanse C.

11. The composition according to claim 10, wherein said polysaccharide binding peptide is CBD.sub.N1.

12. The composition according to claim 9, wherein said phase separation inducing agent is selected from the group consisting of a polyethylene glycol polymer, a dextran, and a copolymer of ethylene oxide and propylene oxide.

13. A composition comprising:

a fusion polypeptide which comprises a non-catalytic polysaccharide binding peptide and a ligand bound to (1) a phase-forming oligosaccharide polymer and (2) a cell having a receptor for said ligand on its surface to which said ligand binds, wherein said composition is obtained by the method of contacting said fusion polypeptide with said cell to form a complex with said cell and contacting said complex with a two-phase partition system which comprises as a first phase a phase-forming oligosaccharide polymer to which said polysaccharide binding peptide binds with a Ka of 10.sup.3 M to 10.sup.7 M, and as a second phase a phase separation inducing agent selected from the group consisting of a polyethylene glycol polymer, a dextran, and a copolymer of ethylene oxide and propylene oxide, and a salt at a concentration of at least 3 M, whereby said complex partitions into said first phase by binding to said phase-forming oligosaccharide polymer; and recovering said first phase, whereby said composition is obtained.

14. The composition according to claim 13, wherein said ligand is a macromolecule.

15. The composition according to claim 14, wherein said macromolecule is a protein.

16. The composition according to claim 14, wherein said macromolecule is selected from the group consisting of an enzyme, a hormone, and an antibody.

17. The composition according to claim 14, wherein said macromolecule is bound to said polysaccharide binding peptide via an amino acid sequence.

18. The composition according to claim 14, wherein said fusion polypeptide comprises a protease recognition sequence between said polysaccharide binding peptide and said macromolecule.

19. The composition according to claim 18, wherein said protease recognition sequence is heterologous to said polysaccharide binding peptide.

20. The composition according to claim 18, wherein said protease recognition sequence is a factor Xa recognition sequence.

21. The composition according to claim 18, wherein said protease recognition sequence is a non-specific protease recognition sequence.

22. A two-phase partition system for affinity separation comprising:

as a first component a phase-forming oligosaccharide polymer to which a polysaccharide binding peptide binds with a Ka of 10.sup.3 M to 10.sup.7 M and as a second component a phase separation inducing agent selected from the group consisting of a dextran, a copolymer of ethylene oxide and propylene oxide, and a salt at a concentration of at least 3 M, wherein said first and second components are each present in an amount sufficient to induce phase separation upon mixing of said first component and said second component, wherein said first component is selected from the group consisting of hydroxyethyl cellulose, carboxymethyl cellulose, methyl cellulose, ethylhydroxyethyl cellulose and hydroxypropyl cellulose.

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L5: Entry 2 of 2 File: USPT Dec 18, 2001

US-PAT-NO: 6331416
DOCUMENT-IDENTIFIER: US 6331416 B1

TITLE: Process of expressing and isolating recombinant proteins and recombinant protein products from plants, plant derived tissues or cultured plant cells

6/10/1999
12/18/2001

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/69.7; 435/252.3, 435/320.1, 435/468, 435/69.1, 530/387.3, 536/23.1, 536/23.4

CLAIMS:

What is claimed is:

1. A process of producing a protein of interest in a plant, plant derived tissue or cultured plant cells and of isolating the protein from the plant, plant derived tissue or cultured plant cells, the process comprising the steps of:

(a) providing a plant, a plant derived tissue or cultured plant cells expressing a fusion protein including the protein of interest and a cellulose binding peptide being fused thereto, said fusion protein being compartmentalized within cells of said plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of said cells of said plant, plant derived tissue or cultured plant cells;

(b) homogenizing said plant, plant derived tissue or cultured plant cells, so as to bring into contact said fusion protein with a plant derived cellulosic matter of said plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of said fusion protein via said cellulose binding peptide to said cellulosic matter, thereby obtaining a fusion protein cellulosic matter complex; and

(c) isolating said fusion protein cellulosic matter complex.

2. The process of claim 1, further comprising the step of:

(d) washing said fusion protein cellulosic matter complex, thereby removing endogenous plant proteins and other plant material therefrom.

3. The process of claim 2, further comprising the step of:

(e) collecting said fusion protein cellulosic matter complex as a final product of the process.

4. The process of claim 2, further comprising the step of:

(e) exposing said fusion protein cellulosic matter complex to conditions effective in dissociating said fusion protein from said cellulosic matter; and

(f) isolating said fusion protein, thereby obtaining an isolated fusion protein.

5. The process of claim 4, wherein said conditions effective in dissociating said fusion protein from said cellulosic matter are selected from the group consisting of basic conditions, denaturative conditions and affinity displacement conditions.

6. The process of claim 4, further comprising the step of:

(g) exposing said isolated fusion protein to conditions effective in digesting said fusion protein so as to release said protein of interest from said fusion protein, thereby obtaining a released protein of interest.

7. The process of claim 6, wherein said conditions effective in digesting said fusion protein so as to release said protein of interest therefrom are selected from the group consisting of proteolysis effected via a protease and proteolysis effected under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

8. The process of claim 6, further comprising the step of:

(h) isolating said released protein of interest.

9. The process of claim 2, further comprising the step of:

(e) exposing said fusion protein cellulosic matter complex to conditions effective in digesting said fusion protein so as to release said protein of interest therefrom, thereby obtaining a released protein of interest.

10. The process of claim 9, wherein said conditions effective in digesting said fusion protein so as to release said protein of interest therefrom are selected from the group consisting of proteolysis effected via a protease and proteolysis effected under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

11. The process of claim 9, further comprising the step of:

(f) isolating said released protein of interest.

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L5: Entry 2 of 2 File: USPT Dec 18, 2001

DOCUMENT-IDENTIFIER: US 6331416 B1
TITLE: Process of expressing and isolating recombinant proteins and recombinant protein products from plants, plant derived tissues or cultured plant cells

Abstract Text (1):
A process of expressing a recombinant protein in a plant and of isolating the recombinant protein from the plant, the process is effected by (a) providing a plant, a plant derived tissue or cultured plant cells expressing a fusion protein including the recombinant protein and a cellulose binding peptide being fused thereto, the fusion protein being compartmentalized within cells of the plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant, plant derived tissue or cultured plant cells; (b) homogenizing the plant, plant derived tissue or cultured plant cells, so as to bring into contact the fusion protein with a cellulosic matter of the plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of the fusion protein via the cellulose binding peptide to the cellulosic matter, thereby obtaining a fusion protein cellulosic matter complex; and (c) isolating the fusion protein cellulosic matter complex.

Brief Summary Text (3):
More particularly, the present invention relates to a process expressing and isolating recombinant proteins and recombinant protein products from plants, plant derived tissue or cultured plant cells, which process employs the expression of a fusion protein including a recombinant protein and a cellulose binding peptide fused thereto, plant homogenization, isolation of a fusion protein cellulosic matter complex and optional subsequent isolation of the fusion protein and/or the recombinant protein from the complex. The present invention further relates to nucleic acid molecules and to genetically modified or viral infected plants or plant cells which are useful while implementing the process, and further to a novel composition of matter which results from the process.

Brief Summary Text (19):
The biolistic or particle gun method, which permits genetic material to be delivered directly into intact cells or tissues by bombarding regeneratable tissues, such as meristems or embryogenic callus, with DNA-coated microparticles has contributed to plant transformation simplicity and efficiency. The microparticles penetrate the plant cells and act as inert carriers of a genetic material to be introduced therein. Microprojectile bombardment of embryogenic suspension cultures has proven successful for the production of transgenic plants of a variety of species. Various parameters that influence DNA delivery by particle bombardment have been defined (Klein et al., Bio/Technology (1998) 6:559-563; McCabe et al., Bio/Technology (1998) 6:923-926; and Sanford, Physiol. Plant. (1990) 79:206-209).

Brief Summary Text (41):
According to one aspect of the present invention there is provided a process of expressing a recombinant protein in a plant and of isolating the recombinant protein from the plant, the process comprising the steps of (a) providing a plant, a plant derived tissue or cultured plant cells expressing a fusion protein including the recombinant protein and a cellulose binding peptide being fused thereto, the fusion protein being compartmentalized within cells of the plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant, plant derived tissue or cultured plant cells; (b) homogenizing the plant, plant derived tissue or cultured plant cells, so as to bring into contact the fusion protein with a cellulosic matter of the plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of the fusion protein via the cellulose binding peptide to the

cellulosic matter, thereby obtaining a fusion protein cellulosic matter complex; and (c) isolating the fusion protein cellulosic matter complex.

Brief Summary Text (42):
According to further features in preferred embodiments of the invention described below, the process further comprising the steps of washing the fusion protein cellulosic matter complex, thereby removing endogenous plant proteins and other plant material therefrom and collecting the fusion protein cellulosic matter complex as a final product of the process.

Brief Summary Text (43):
According to still further features in the described preferred embodiments the process further comprising the steps of washing the fusion protein cellulosic matter complex, thereby removing endogenous plant proteins and other plant material therefrom, exposing the fusion protein cellulosic matter complex to conditions effective in dissociating the fusion protein from the cellulosic matter; and isolating the fusion protein, thereby obtaining an isolated fusion protein.

Brief Summary Text (44):
According to still further features in the described preferred embodiments the process further comprising the steps of exposing the isolated fusion protein to conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom, thereby obtaining a released recombinant protein and isolating the released recombinant protein.

Brief Summary Text (45):
According to still further features in the described preferred embodiments the process further comprising the steps of washing the fusion protein cellulosic matter complex, thereby removing endogenous plant proteins and other plant material therefrom, exposing the fusion protein cellulosic matter complex to conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom, thereby obtaining a released recombinant protein, and isolating the released recombinant protein.

Brief Summary Text (46):
According to still further features in the described preferred embodiments, the conditions effective in dissociating the fusion protein from the cellulosic matter are selected from the group consisting of basic conditions, denaturative conditions and affinity displacement conditions.

Brief Summary Text (47):
According to still further features in the described preferred embodiments, the conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom are selected from the group consisting of proteolysis effected via a protease and proteolysis effected under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

Brief Summary Text (48):
According to another aspect of the present invention there is provided a genetically modified or viral infected plant or cultured plant cells expressing a fusion protein including a recombinant protein and a cellulose binding peptide.

Brief Summary Text (49):
According to further features in preferred embodiments of the invention described below, the fusion protein is compartmentalized within cells of the plant or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant or cultured plant cells.

Brief Summary Text (50):
According to still further features in the described preferred embodiments the fusion protein is compartmentalized within a cellular compartment selected from the group consisting of cytoplasm, endoplasmic reticulum, golgi apparatus, oil bodies, starch bodies, chloroplastids, chloroplasts, chromoplastids, chromoplasts, vacuole, lysosomes, mitochondria, and nucleus.

Brief Summary Text (51):
According to still further features in the described preferred embodiments expression of the

fusion protein is under a control of a constitutive or tissue specific plant promoter.

Brief Summary Text (52):
According to still further features in the described preferred embodiments the fusion protein includes a recombinant protein and a cellulose binding peptide separated therebetween via a unique amino acid sequence recognizable and digestible by a protease or under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

Brief Summary Text (53):
According to yet another aspect of the present invention there is provided a composition of matter comprising (a) a plant derived cellulosic matter; and (b) a fusion protein including a recombinant protein and a cellulose binding peptide separated therebetween via a unique amino acid sequence recognizable and digestible by a protease or under predetermined cis or trans conditions for digesting a controllable intervening protein sequence, the fusion protein being complexed to the plant derived cellulosic matter by affinity binding via the cellulose binding peptide.

Detailed Description Text (7):
The process according to the present invention is directed at expressing a recombinant protein in a plant and further at isolating the recombinant protein from the plant. The process according to the present invention is effected by first providing a plant, a plant derived tissue or cultured plant cells (which are referred to herein below individually and collectively as "plant material") 10 expressing a fusion protein which includes the recombinant protein and a cellulose binding peptide fused thereto. The fusion protein is compartmentalized within cells of the plant material, so as to be sequestered from cell walls of the cells of the plant material. As used herein in the specification and in the claims section that follows, the phrase "cultured plant cells" includes both non-differentiated plant cell cultures and some what more differentiated callus cultures.

Detailed Description Text (8):
Compartmentalizing and thereby sequestering the fusion protein from the cell walls is an essential feature of the present invention because high levels of expressed cellulose binding peptide associated with plant cell walls inhibit plant growth to a great extent. See to this effect U.S. patent applications Ser. Nos. 09/006,632; 09/006,636; and PCT/IL98/00345 (WO 99/07830).

Detailed Description Text (9):
When sufficient expression has been detected by sampling and testing the plant material as further detailed hereinunder, the plant material is homogenized 12 so as to bring into contact the fusion protein with a cellulosic matter of the plant material, to thereby effect affinity binding 14 of the fusion protein via the cellulose binding peptide to the cellulosic matter, thereby obtaining a fusion protein cellulosic matter complex. Conditions such as, but not limited to, temperature, pH, salt concentration, time and the like are preferably set so as to allow maximal binding. Such conditions are well know to the skilled artisan and can be experimentally modified to best suit a specific application. Sampling and testing can be employed to monitor the binding process, as further detailed hereinunder.

Detailed Description Text (10):
When sufficient binding has occurred the fusion protein cellulosic matter complex is collected or isolated 16 by methods well known to the skilled artisan which method are traditionally employed for isolation of cellulosic matter from plant material. Thereafter, a wash step 18 is employed to remove unbound material, including, in particular, unbound endogenous plant proteins, thereby isolating the fusion protein cellulosic matter complex. The wash step can be repeated one or several times with a single or several wash solutions, each of which can include in addition to water, buffers, salts, detergents and the like to efficiently effect the removal of unbound matter from the fusion protein cellulosic matter complex. The wash step can be effected in solution using appropriate stiring, however, advantageously, the wash step is effected within a column into which the collected or isolated fusion protein cellulosic matter complex is packed and subsequently washed.

Detailed Description Text (11):
According to one embodiment of the present invention, and as indicated in FIG. 1 by numeral 20,

the fusion protein cellulosic matter complex is collected as a final product of the process. Such a final product can serve as a pack for affinity columns. In this case the recombinant protein is selected to have affinity to a ligand, which can then be affinity purified via a column packed with the fusion protein cellulosic matter complex, in a manner otherwise similar to that described in U.S. Pat. No. 5,474,925, which is incorporated herein by reference. One of the advantages of the process described herein over the teachings of U.S. Pat. No. 5,474,925 is that by sequestering the fusion protein from the cell walls one can achieve very high expression of the fusion protein as compared to the low expression levels practically enabled by U.S. Pat. No. 5,474,925, because no deleterious effect on plant growth is exerted. As a result, the specific activity of the fusion protein cellulosic matter complex formed according to the present invention, i.e., the number of fusion protein molecules per weight of cellulosic matter, is far superior. Further details relating to the effect of high cellulose binding peptide expression on plant development see also PCT/IL98/000345.

Detailed Description Text (12):
According to another embodiment of the present invention, as indicated by numeral 22, the final product of the process according to the present invention is the fusion protein itself 24. Thus, according to this embodiment of the present invention, conditions effective in dissociating the fusion protein from the cellulosic matter are used to effect such dissociation. The dissociated fusion protein is thereafter readily isolated by any conventional separation technique, such as, but not limited to, elution or size separation, such as differential filtration or centrifugation, thereby obtaining an isolated fusion protein. Conditions effective in dissociating the fusion protein from the cellulosic matter include, but are not limited to, basic conditions (e.g., 20 mM Tris pH 12) which are known to dissociate all cellulose binding peptides from cellulose, denaturative conditions, or affinity displacement conditions, e.g., using 200 nM glucose or cellobiose which are know in their ability to elute family IX cellulose binding domains (CBDs). Alternatively, a protein cleavage site can be inserted in the cellulose binding peptide to facilitate the dissociation of the fusion protein by specific proteolysis, for example. See to this effect and to other uses of CBD-fusion proteins U.S. Pat. Nos. 5,719,044; 5,670,623; 5,856,021; 5,137,819; 5,202,247; 5,340,731; and 5,474,925; and U.S. patent applications Ser. Nos. 08/788,621; and 08/788,622; EP 0 381 719 B1, and EP application No. 93907724.4. See also the teachings of U.S. Pat. No. 5,834,247, which is further described hereinunder.

Detailed Description Text (13):
As indicated by numeral 26, the fusion protein thus isolated can be exposed to conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom, thereby obtaining a released recombinant protein which can be thereafter isolated as a final product 28. Conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom include, but are not limited to, proteolysis effected via a protease, such as, but not limited to, Factor Xa, enterokinase, thrombin, trypsin, papain, pepsin, chemotrypsin and the like, or proteolysis effected via controllable intervening protein sequence (CIVPS) inserted into or adjacent the cellulose binding peptide, the CIVPS are capable of excision from or cleavage of the peptide under predetermined conditions in cis or in trans, e.g., increase in temperature, exposure to light, unblocking of amino acid residues by dephosphorylation and treatment with chemical reagents or deglycosylation, examples include proteolysis effected under acidic conditions (HCl, e.g., to cleave between Asp and Pro) and proteolysis effected by a proteolysing reagent, such as CNBr to cleave downstream of Met, all as known in the art and/or as further described in U.S. Pat. No. 5,834,247, which is incorporated herein by reference.

Detailed Description Text (15):
According to an alternative embodiment of the process of the present invention, as indicated in FIG. 1 by numeral 30, the washed fusion protein cellulose matter complex resulting from step 18 is exposed to conditions effective in digesting the fusion protein so as to release the recombinant protein therefrom, thereby obtaining a released recombinant protein. These conditions are similar to those described with respect to step 26. The released recombinant protein is thereafter readily isolated by any conventional separation technique, such as, but not limited to, displacement or size separation, such as differential filtration or centrifugation, thereby obtaining an isolated recombinant protein final product 32.

Detailed Description Text (18):

Such peptides include amino acid sequences expressible in plants that are originally derived from a cellulose binding region of, e.g., a cellulose binding protein (CBP) or a cellulose binding domain (CBD). The cellulose binding peptide according to the present invention can include any amino acid sequence expressible in plants which binds to a cellulose polymer. For example, the cellulose binding domain or protein can be derived from a cellulase, a binding domain of a cellulose binding protein or a protein screened for, and isolated from, a peptide library, or a protein designed and engineered to be capable of binding to cellulose or to saccharide units thereof, and which is expressible in plants. The cellulose binding domain or protein can be naturally occurring or synthetic, as long as it is expressible in plants. Suitable polysaccharidases from which a cellulose binding domain or protein expressible in plants may be obtained include .beta.-1,4-glucanases. In a preferred embodiment, a cellulose binding domain or protein from a cellulase or scaffoldin is used. Typically, the amino acid sequence of the cellulose binding peptide expressed in plants according to the present invention is essentially lacking in the hydrolytic activity of a polysaccharidase (e.g., cellulase, chitinase), but retains the cellulose binding activity. The amino acid sequence preferably has less than about 10% of the hydrolytic activity of the native polysaccharidase; more preferably less than about 5%, and most preferably less than about 1% of the hydrolytic activity of the native polysaccharidase, ideally no activity altogether.

Detailed Description Text (19):
The cellulose binding domain or protein can be obtained from a variety of sources, including enzymes and other proteins which bind to cellulose which find use in the subject invention.

Detailed Description Text (20):
In Table 4 below are listed those binding domains which bind to one or more soluble/insoluble polysaccharides including all binding domains with affinity for soluble glucans (.alpha., .beta., and/or mixed linkages). The N1 cellulose-binding domain from endoglucanase CenC of C. fimi is the only protein known to bind soluble cellosaccharides and one of a small set of proteins which are known to bind any soluble polysaccharides. Also, listed in Tables 1 to 3 are examples of proteins containing putative .beta.-1,3-glucan-binding domains (Table 1); proteins containing Streptococcal glucan-binding repeats (Cp1 superfamily) (Table 2); and enzymes with chitin-binding domains, which may also bind cellulose (Table 3). The genes encoding each one of the peptides listed in Tables 1-4 are either isolated or can be isolated as further detailed hereinunder, and therefore, such peptides are expressible in plants. Scaffoldin proteins or portions thereof, which include a cellulose binding domain, such as that produced by Clostridium cellulovorans (Shoseyov et al., PCT/US94/04132) can also be used as the cellulose binding peptide expressible in plants according to the present invention. Several fungi, including Trichoderma species and others, also produce polysaccharidases from which polysaccharide binding domains or proteins expressible in plants can be isolated. Additional examples can be found in, for example, Microbial Hydrolysis of Polysaccharides, R. A. J. Warren, Annu. Rev. Microbiol. 1996, 50:183-212; and "Advances in Microbial Physiology" R. K. Poole, Ed., 1995, Academic Press Limited, both are incorporated by reference as if fully set forth herein.

Detailed Description Text (22):
The K.sub.a for binding of the cellulose binding domains or proteins to cellulose is at least in the range of weak antibody-antigen extractions, i.e., .gtoreq.10.sup.3, preferably 10.sup.4, most preferably 10.sup.6 M.sup.-1. If the binding of the cellulose binding domain or protein to cellulose is exothermic or endothermic, then binding will increase or decrease, respectively, at lower temperatures, providing a means for temperature modulation of the binding step, see numeral 14 in FIG. 1.

Detailed Description Text (26):
Probes developed using consensus sequences for the binding domain of a polysaccharidase or polysaccharide-binding protein are of particular interest. The .beta.-1,4-glycanases from C. fimi characterized to date are endoglucanases A, B, C and D (CenA, CenB, CenC and CenD, respectively), exocellobiohydrolases A and B (CbhA and CbhB, respectively), and xylanases A and D (Cex and XylD, respectively) (see Wong et al. (1986) Gene, 44:315; Meinke et al. (1991) J. Bacteriol., 173:308; Coutinho et al., (1991) Mol. Microbiol. 5:1221; Meinke et al., (1993) Bacteriol., 175:1910; Meinke et al., (1994) Mol. Microbiol., 12:413; Shen et al., Biochem. J., in press; O'Neill et al., (1986) Gene, 44:325; and Millward-Sadler et al., (1994) Mol. Microbiol., 11:375). All are modular proteins of varying degrees of complexity, but with two

features in common: a catalytic domain (CD) and a cellulose-binding domain (CBD) which can function independently (see Millward-Sadler et al., (1994) Mol. Microbiol., 11:375; Gilkes et al., (1988) J. Biol. Chem., 263:10401; Meinke et al., (1991) J. Bacteriol., 173:7126; and Coutinho et al., (1992) Mol. Microbiol., 6:1242). In four of the enzymes, CenB, CenD, CbhA and CbhB, fibronectin type III (Fn3) repeats separate the N-terminal CD from the C-terminal CBD. The CDs of the enzymes come from six of the families of glycoside hydrolases (see Henrissat (1991) Biochem. J., 280:309; and Henrissat et al., (1993) Biochem. J., 293:781); all of the enzymes have an N- or C-terminal CBD or CBDs (see Tomme et al., Adv. Microb. Physiol., in press); CenC has tandem CBDs from family IV at its N-terminus; CenB and XylD each have a second, internal CBD from families III and II, respectively. Cex and XylD are clearly xylanases; however, Cex, but not XylD, has low activity on cellulose. Nonetheless, like several other bacterial xylanases (see Gilbert et al., (1993) J. Gen. Microbiol., 139:187), they have CBDs. C. fimi probably produces other .beta.-1,4-glycanases. Similar systems are produced by related bacteria (see Wilson (1992) Crit. Rev. Biotechnol., 12:45; and Hazlewood et al., (1992) J. Appl. Bacteriol., 72:244). Unrelated bacteria also produce glycanases; Clostridium thermocellum, for example, produces twenty or more .beta.-1,4-glycanases (see Beguin et al., (1992) FEMS Microbiol. Lett., 100:523). The CBD derived from C. fimi endoglucanase C N1, is the only protein known to bind soluble cellosaccharides and one of a small set of proteins that are known to bind any soluble polysaccharides.

Detailed Description Text (30):
Any cellulose binding protein or cellulose binding domain may be used in the present invention. The term "cellulose binding protein" ("CBP") refers to any protein or polypeptide which specifically binds to cellulose. The cellulose binding protein may or may not have cellulose or cellulolytic activity. The term "cellulose binding domain" ("CBD") refers to any protein or polypeptide which is a region or portion of a larger protein, said region or portion binds specifically to cellulose. The cellulose binding domain (CBD) may be a part or portion of a cellulase, xylanase or other polysaccharidase, e.g., a chitinase, etc., a sugar binding protein such as maltose binding protein, or scaffoldin such as CbpA of Clostridium celluvorans, etc. Many cellulases and hemicellulases (e.g. xylanases and mannases) have the ability to associate with cellulose. These enzymes typically have a catalytic domain containing the active site for substrate hydrolysis and a carbohydrate-binding domain or cellulose-binding domain for binding cellulose. The CBD may also be from a non-catalytic polysaccharide binding protein. To date, more than one hundred cellulose-binding domains (CBDs) have been classified into at least thirteen families designated I-XIII (Tomme et al. (1995) "CelluloseBinding Domains: Classification and Properties", in ACS Symposium Series 618 Enzymatic Degradation and Insoluble Carbohydrates, pp. 142-161, Saddler and Penner eds., American Chemical Society, Washington, D.C. (Tomme I); Tomme et al. Adv. Microb. Physiol. (1995) 37:1 (Tomme II); and Smant et al., Proc. Natl. Acad. Sci U.S.A. (1998) 95:4906,-4911, all of which are incorporated herein by reference). Any of the CBDs described in Tomme I or II or any variants thereof, any other presently known CBDs or any new CBDs which may be identified can be used in the present invention. As an illustrative, but in no way limiting example, the CBP or CBD can be from a bacterial, fungal, slime mold, or nematode protein or polypeptide. For a more particular illustrative example, the CBD is obtainable from Clostridium cellulovorans, Clostridium cellulovorans, or Cellulomonas fimi (e.g., CenA, CenB, CenD, Cex). In addition, the CBD may be selected from a phage display peptide or peptidomimetic library, random or otherwise, using e.g., cellulose as a screening agent. (See Smith Science (1985) 228:1315-1317 and Lam, Nature (1991) 354:82-84). Furthermore, the CBD may be derived by mutation of a portion of a protein or polypeptide which binds to a polysaccharide other than cellulose (or hemicellulose) but also binds cellulose, such as a chitinase, which specifically binds chitin, or a sugar binding protein such as maltose binding protein, rendering said portion capable of binding to cellulose. In any event, the CBD binds cellulose or hemicellulose. Shoseyov and Doi (Proc. Natl. Acad. Sci. USA (1990) 87:2192-2195) isolated a unique cellulose-binding protein (CbpA) from the cellulose "complex" of the cellulolytic bacterium Clostridium cellulovorans. This major subunit of the cellulose complex was found to bind to cellulose, but had no hydrolytic activity, and was essential for the degradation of crystalline cellulose. The CbpA gene has been cloned and sequenced (Shoseyov et al. Proc. Natl. Acad. Sci. USA (1992) 89:3483-3487). Using PCR primers flanking the cellulose-binding domain of CbpA, the latter was successfully cloned into an overexpression vector that enabled overproduction of the approximately 17 kDa CBD in Escherichia coli. The recombinant CBD exhibits very strong affinity to cellulose and chitin (U.S. Pat. No. 5,496,934; Goldstein et al., J. Bacteriol. (1993) 175:5762; PCT International Publication WO 94/24158, all are incorporated by reference as if fully set forth

herein).

Detailed Description Text (31):
In recent years, several CBDs have been isolated from different sources. Most of these have been isolated from proteins that have separate catalytic, i.e., cellulose and cellulose binding domains, and only two have been isolated from proteins that have no apparent hydrolytic activity but possess cellulose-binding activity (Goldstein et al. J. Bacteriol. (1993) 175:5762-5768; Morag et al. Appl. (1995) Environ. Microbiol. 61:1980-1986).

Detailed Description Text (52):
Other recombinant proteins of interest, will for the most part be mammalian proteins, and will include blood proteins, such as serum albumin, Factor VII, Factor VIIIc, Factor VIIIvW, Factor IX, Factor X, tissue plasminogen factor, Protein C, von Willebrand factor, antithrombin III, erythropoietin, colony stimulating factors, such as G-, M-, GM-, cytokines, such as interleukins 1-11, integrins, addressing, selecting, homing receptors, surface membrane proteins, such as surface membrane protein receptors, T cell receptor units, immunoglobulins (as further detailed above with respect to antibodies), soluble major histocompatibility complex antigens, structural proteins, such as collagen, fibrin, elastin, tubulin, actin, and myosin, growth factor receptors, growth factors, growth hormone, cell cycle proteins, vaccines, fibrinogen, thrombin, cytokines and hyaluronidase. Additional examples include chymosin, polymerases, saccharidases, dehydrogenases, nucleases, oxido reductases such as fungal peroxidases and lactases, xylanases, rennin, horse radish peroxidase, amylases and soil remediation enzymes.

Detailed Description Text (55):
The fusion of two proteins for which genes has been isolated is well known and practiced in the art. Such fusion involves the joining together of heterologous nucleic acid sequences, in frame, such that translation thereof results in the generation of a fused protein product or a fusion proteins. Methods, such as the polymerase chain reaction (PCR), restriction, nuclease digestion, ligation, synthetic oligonucleotides synthesis and the like are typically employed in various combinations in the process of generating fusion gene constructs. One ordinarily skilled in the art can readily form such constructs for any pair or more of individual proteins. Interestingly, in most cases where such fusion or chimera proteins are produced, and in all cases where one of the proteins was a cellulose binding peptide, both the former and the latter retained their catalytic activity or function.

Detailed Description Text (56):
For example, Greenwood et al. (1989, FEBS Lett. 224:127-131) fused the cellulose binding region of Cellulomonas fimi endoglucanase to the enzyme alkaline phosphatase. The recombinant fusion protein retained both its phosphatase activity and the ability to bind to cellulose. For more descriptions of cellulose binding fusion proteins, see U.S. Pat. No. 5,137,819 issued to Kilburn et al., and U.S. Pat. No. 5,719,044 issued to Shoseyov et al. both incorporated by reference herein. See also U.S. Pat. No. 5,474,925. All of which are incorporated herein by reference.

Detailed Description Text (59):
According to a preferred embodiment of the present invention the fusion protein includes the recombinant protein and the cellulose binding peptide separated therebetween via a unique amino acid sequence recognizable and digestible by a protease or under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

Detailed Description Text (60):
As used herein in the specification and in the claims section that follows, the phrase "unique amino acid sequence recognizable and digestible by a protease" includes a protease recognition sequence which is both recognizable and readily accessible to a protease. Thus, the unique sequence can be a solitary sequence (i.e., which does not appear in the recombinant protein and optionally also not in the cellulose binding peptide) or alternatively, the sole sequence of several similar sequences which is not sequestered from the protease due to the tertiary structure of the recombinant protein and optionally the cellulose binding peptide. In both these cases proteolysis will release the recombinant protein from the fusion protein cellulosic matter complex.

Detailed Description Text (62):
Thus, according to an aspect of the present invention there is provided a composition of matter comprising (a) a plant derived cellulosic matter; and (b) a fusion protein including a recombinant protein and a cellulose binding peptide separated therebetween via a unique amino acid sequence recognizable and digestible by a protease or under predetermined cis or trans conditions for digesting a controllable intervening protein sequence, wherein the fusion protein is complexed to the plant derived cellulosic matter by affinity binding via the cellulose binding peptide.

Detailed Description Text (63):
Nucleic acid molecules which can be used according to preferred embodiments of the present invention to express the fusion protein in plant cells would therefore include a heterologous nucleic acid sequence including (i) a first sequence encoding a cellulose binding peptide; (ii) a second sequence encoding a recombinant protein, wherein the first and second sequences are joined together in frame in either orientation; and (iii) a third sequence encoding a unique amino acid sequence recognizable and digestible by a protease or under predetermined cis or trans conditions for digesting a controllable intervening protein sequence, the third sequence is between and in frame with the first and second sequences.

Detailed Description Text (64):
Thus, specific cleavage can be used to release the recombinant protein from the fusion protein cellulosic matter complex. For example, one can include a protease recognition site or a chemical cleavage site between the recombinant protein and the cellulose binding peptide. Examples of recognition sites include those for collagenase, thrombin, enterokinase, and Factor X.sub.a which are cleaved specifically by the respective enzymes. Chemical cleavage sites sensitive, for example, to low pH or cyanogen bromide, can also be used.

Detailed Description Text (74):
Following transformation plant propagation is exercised. The most common method of plant propagation is by seed. Regeneration by seed propagation, however, has the deficiency that due to heterozygosity there is a lack of uniformity in the crop, since seeds are produced by plants according to the genetic variances governed by Mendelian rules. Basically, each seed is genetically different and each will grow with its own specific traits. Therefore, it is preferred that the transgenic plant be produced such that the regenerated plant has the identical traits and characteristics of the parent transgenic plant, e.g., a reproduction of the fusion protein. Therefore, it is preferred that the transgenic plant be regenerated by micropropagation which provides a rapid, consistent reproduction of the transgenic plants.

Detailed Description Text (75):
Micropropagation is a process of growing new generation plants from a single piece of tissue that has been excised from a selected parent plant or cultivar. This process permits the mass reproduction of plants having the preferred tissue expressing the fusion protein. The new generation plants which are produced are genetically identical to, and have all of the characteristics of, the original plant. Micropropagation allows mass production of quality plant material in a short period of time and offers a rapid multiplication of selected cultivars in the preservation of the characteristics of the original transgenic or transformed plant. The advantages of cloning plants are the speed of plant multiplication and the quality and uniformity of plants produced.

Detailed Description Text (80):
The constructs of the subject invention will include an expression cassette for expression of the fusion protein of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous sequence one or more of the following sequence elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

Detailed Description Text (87):
In one embodiment, a plant viral nucleic acid is provided in which the native coat protein coding sequence has been deleted from a viral nucleic acid, a non-native plant viral coat

protein coding sequence and a non-native promoter, preferably the subgenomic promoter of the non-native coat protein coding sequence, capable of expression in the plant host, packaging of the recombinant plant viral nucleic acid, and ensuring a systemic infection of the host by the recombinant plant viral nucleic acid, has been inserted. Alternatively, the coat protein gene may be inactivated by insertion of the non-native nucleic acid sequence within it, such that a fusion protein is produced. The recombinant plant viral nucleic acid may contain one or more additional non-native subgenomic promoters. Each non-native subgenomic promoter is capable of transcribing or expressing adjacent genes or nucleic acid sequences in the plant host and incapable of recombination with each other and with native subgenomic promoters. Non-native (foreign) nucleic acid sequences may be inserted adjacent the native plant viral subgenomic promoter or the native and a non-native plant viral subgenomic promoters if more than one nucleic acid sequence is included. The non-native nucleic acid sequences are transcribed or expressed in the host plant under control of the subgenomic promoter to produce the desired products.

Detailed Description Text (91):
The viral vectors are encapsidated by the coat proteins encoded by the recombinant plant viral nucleic acid to produce a recombinant plant virus. The recombinant plant viral nucleic acid or recombinant plant virus is used to infect appropriate host plants. The recombinant plant viral nucleic acid is capable of replication in the host, systemic spread in the host, and transcription or expression of foreign gene(s) in the host to produce the desired fusion protein.

Detailed Description Text (92):
Fusion protein compartmentalization--signal peptides:

Detailed Description Text (93):
As already mentioned hereinabove, compartmentalization of the fusion protein is an important feature of the present invention because it allows undisturbed plant growth. Thus, according to one aspect of the present invention, the fusion protein is compartmentalized within cells of the plant or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant or cultured plant cells.

Detailed Description Text (94):
The fusion protein can be compartmentalized within a cellular compartment, such as, for example, the cytoplasm, endoplasmic reticulum, golgi apparatus, oil bodies, starch bodies, chloroplastids, chloroplasts, chromoplastids, chromoplasts, vacuole, lysosomes, mitochondria or the nucleus.

Detailed Description Text (96):
The following provides description of signal peptides which can be used to direct the fusion protein according to the present invention to specific cell compartments.

Detailed Description Text (97):
It is well-known that signal peptides serve the function of translocation of produced protein across the endoplasmic reticulum membrane. Similarly, transmembrane segments halt translocation and provide anchoring of the protein to the plasma membrane, see, Johnson et al. The Plant Cell (1990) 2:525-532; Sauer et al. EMBO J. (1990) 9:3045-3050; Mueckler et al. Science (1985) 229:941-945. Mitochondrial, nuclear, chloroplast, or vacuolar signals target expressed protein correctly into the corresponding organelle through the secretory pathway, see, Von Heijne, Eur. J. Biochem. (1983) 133:17-21; Yon Heijne, J. Mol. Biol. (1986) 189:239-242; Iturriaga et al. The Plant Cell (1989) 1:381-390; McKnight et al., Nucl. Acid Res. (1990) 18:4939-4943; Matsuoka and Nakamura, Proc. Natl. Acad. Sci. USA (1991) 88:834-838. A recent book by Cunningham and Porter (Recombinant proteins from plants, Eds. C. Cunningham and A. J. R. Porter, 1998 Humana Press Totowa, N.J.) describe methods for the production of recombinant proteins in plants and methods for targeting the proteins to different compartments in the plant cell. In particular, two chapters therein (14 and 15) describe different methods to introduce targeting sequences that results in accumulation of recombinant proteins in compartments such as ER, vacuole, plastid, nucleus and cytoplasm. The book by Cunningham and Porter is incorporated herein by reference. Presently, the preferred site of accumulation of the fusion protein according to the present invention is the ER using signal peptide such as Cel 1 or the rice amylase signal peptide at the N-terminus and an ER retaining peptide (HDEL, SEQ ID NO:1; or KDEL, SEQ ID NO:2)

at the C-terminus.

Detailed Description Text (99):
Any promoter which can direct the expression of the fusion protein according to the present invention can be utilized to implement the process of the instant invention, both constitutive and tissue specific promoters. According to presently preferred embodiment the promoter selected is constitutive, because such a promoter can direct the expression of higher levels of the fusion protein. In this respect the present invention offers a major advantage over the teachings of U.S. Pat. No. 5,474,925 in which only tissue specific and weak promoters can be employed because of the detelerious effect of the fusion protein described therein on cell wall development. The reason for which the present invention can utilize strong and constitutive promoters relies in the compartmentalization and sequestering approach which prohibits contact between the expressed fusion protein and the plant cell walls which such walls are developing.

Detailed Description Text (108):
Expression of the fusion protein can be monitored by a variety of methods. For example, ELISA or western blot analysis using antibodies specifically recognizing the recombinant protein or its cellulose binding peptide counterpart can be employed to qualitatively and/or quantitatively monitor the expression of the fusion protein in the plant. Alternatively, the fusion protein can be monitored by SDS-PAGE analysis using different staining techniques, such as, but not limited to, coomasie blue or silver staining. Other methods can be used to monitor the expression level of the RNA encoding for the fusion protein. Such methods include RNA hybridization methods, e.g., Northern blots and RNA dot blots.

Detailed Description Text (109):
Binding of the fusion protein to the plant derived cellulosic matter:

Detailed Description Text (110):
When sufficient expression has been detected, binding of the fusion protein to the plant derived cellulosic matter is effected. Such binding can be achieved, for example, as follows. Whole plants, plant derived tissue or cultured plant cells are homogenized by mechanical method in the presence or absence of a buffer, such as, but not limited to, PBS. The fusion protein is therefore given the opportunity to bind to the plant derived cellulosic matter. Buffers that may include salts and/or detergents at optimal concentrations may be used to wash non specific proteins from the cellulosic matter.

Detailed Description Text (112):
In general, a recent book by Cunningham and Porter (Recombinant proteins from plants, Eds. C. Cunningham and A. J. R. Porter, 1998 Humana Press Totowa, N.J.) describes methods for the production of recombinant proteins in plants including methods for extraction of the proteins from the plants. The methods used herein for extraction of proteins from plants are similar, however the ability of the fusion protein to bind to cellulose dictates its fate, unless extraction is done under condition in which the cellulose binding peptide do not bind to cellulose, for example, pH higher than 10 (for most CBDs) or high concentration of glucose or cellobiose (200 mM or higher) for family IX CBDs. If the initial extraction is conducted under conditions that prevent binding, the supernatant is cleared from the cellulosic matter and then the solution is brought by either dilution, dialysis or pH correction, if necessary, to a condition that enables binding, after which cellulose is added in a batch or the solution is loaded on a cellulose column. Cellulose affinity purification is conducted as described, for example, in U.S. Pat. Nos. 5,719,044; 5,670,623; 5,856,021; 5,137,819; 5,202,247; 5,340,731; and 5,474,925; and U.S. patent application Ser. No. 08/788,621; and 08/788,622; EP 0 381 719 B1, and EP application No. 93907724.4. Alternatively, the extraction solution provides conditions that favor binding to the plant derived cellulosic matter.

Detailed Description Text (113):
In any case, while the fusion protein is bound to cellulose, further whases can be employed for further removal of unbound proteins, conditions which dissociate such binding or proteolytic cleavage can be used to isolate the fusion protein itself, or proteolytic cleavage can be used to isolate the recombinant protein, all as further detailed hereinabove.

Detailed Description Text (121):
Construction of transgenic plants:

Detailed Description Text (122):
The binary vector was mobilized into disarmed LB 4404 Agrobacterium tumefaciens (An, 1987, Meth. Enzymol. 153:292-305). Tuber discs transformation was performed with Solanum tuberosum cv Desiree plants as described previously (Olesinski et al., 1996, 1996, Plant Phisiol. 111:541-550). Regenerated transgenic plants were selected on kanamycin.

Detailed Description Text (123):
Analysis of transgenic plants:

Detailed Description Text (125):
Transgenic plants transformed with the ProtL-CBD construct can be tested for expression of ProtL-CBD. Thus, for example, a Northern blot analysis of ProtL-CBD RNA expression can be carried out using the DNA fragment of SEQ ID NO:3 or a portion thereof as a probe. Furthermore, RT-PCR can be performed on RNA samples obtained from such transgenic plants using PCR primers No. 3 and No. 4 to amplify and detect the ProtL-CBD RNA. Additional methods for analysis of expression in transgenic plants include RNase protection assays. Expression of the ProtL-CBD fusion protein can alternatively or additionally be evaluated by monitoring the fusion protein itself using, for example, ELISA or Western blot protocols and anti-CBD or ProtL antibodies. Yet in addition, the ProtL-CBD fusion protein can be visualized by SDS-PAGE analysis using different staining techniques, such as coomasie blue or silver staining, immuno precipitation, enzyme linked immunoassays or CBD and ProtL binding assays. In addition, techniques such as in situ hybridization and immunostaning also may be used to detect the presence or expression of the recombinant construct in specific plant organs and tissues. The methods for performing all of these assays are well known to those skilled in the art.

Detailed Description Paragraph Table (4):
TABLE 4 Sources of polysaccharide binding domains Proteins Where Binding Binding Domain Domain is Found Cellulose Binding .beta.-glucanases (avicelases, CMCases, Domains.sup.1 cellodextrinases) exoglucanses or cellobiohydrolases cellulose binding proteins xylanases mixed xylanases/glucanases esterases chitinases .beta.-1,3-glucanases .beta.-1,3-(.beta.-1,4)-glucanases (.beta.-)mannanases .beta.-glucosidases/galactosidases cellulose synthases (unconfirmed) Starch/Maltodextrin .alpha.-amylases.sup.2,3 Binding Domains .beta.-amylases.sup.4,5 pullulanases glucoamylases.sup.6,7 cyclodextrin glucotransferases.sup.8-10 (cyclomaltodextrin glucanotransferases) maltodextrin binding proteins.sup.11 Dextran Binding Domains (Streptococcal) glycosyl transferases.sup.12 dextran sucrases (unconfirmed) Clostridial toxins.sup.13,14 glucoamylases.sup.6 dextran binding proteins .beta.-Glucan Binding Domains .beta.-1,3-glucanases.sup.15,16 .beta.01,3-(.beta.-1,4)-glucanases (unconfirmed) .beta.-1,3-glucan binding protein.sup.17 Chitin Binding Domains chitinases chitobiases chitin binding proteins (see also cellulose binding domains) Heivein .sup.1 Gilkes et al., Adv. Microbiol Reviews, (1991) 303-315. .sup.2 S?gaard et al., J. Biol. Chem. (1993) 268:22480. .sup.3 Weselake et al., Cereal Chem. (1983) 60:98. .sup.4 Svensson et al., J. (1989) 264:309. .sup.5 Jespersen et al., J. (1991) 280:51. .sup.6 Belshaw et al., Eur. J. Biochem. (1993) 211:717. .sup.7 Sigurskjold et al., Eur. J. Biochem. (1994) 225:133. .sup.8 Villette et al., Biotechnol. Appl. Biochem. (1992) 16:57. .sup.9 Fukada et al., Biosci. Biotechnol. Biochem. (1992) 56:556. .sup.10 Lawson et al., J. Mol. Biol. (1994) 236:590. .sup.14 von Eichel-Streiber et al., Mol. Gen. Genet. (1992) 233:260. .sup.15 Klebl et al., J. Bacteriol. (1989) 171:6259. .sup.16 Watanabe et al., J. Bacteriol. (1992) 174:186. .sup.17 Duvic et al., J. Biol. Chem. (1990) :9327.

CLAIMS:

1. A process of producing a protein of interest in a plant, plant derived tissue or cultured plant cells and of isolating the protein from the plant, plant derived tissue or cultured plant cells, the process comprising the steps of:

(a) providing a plant, a plant derived tissue or cultured plant cells expressing a fusion protein including the protein of interest and a cellulose binding peptide being fused thereto, said fusion protein being compartmentalized within cells of said plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of said cells of said plant, plant derived tissue or cultured plant cells;

(b) homogenizing said plant, plant derived tissue or cultured plant cells, so as to bring into contact said fusion protein with a plant derived cellulosic matter of said plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of said fusion protein via said cellulose binding peptide to said cellulosic matter, thereby obtaining a fusion protein cellulosie matter complex; and

(c) isolating said fusion protein cellulosic matter complex.

2. The process of claim 1, further comprising the step of:

(d) washing said fusion protein cellulosic matter complex, thereby removing endogenous plant proteins and other plant material therefrom.

3. The process of claim 2, further comprising the step of:

(e) collecting said fusion protein cellulosic matter complex as a final product of the process.

4. The process of claim 2, further comprising the step of:

(e) exposing said fusion protein cellulosic matter complex to conditions effective in dissociating said fusion protein from said cellulosic matter; and

(f) isolating said fusion protein, thereby obtaining an isolated fusion protein.

5. The process of claim 4, wherein said conditions effective in dissociating said fusion protein from said cellulosic matter are selected from the group consisting of basic conditions, denaturative conditions and affinity displacement conditions.

6. The process of claim 4, further comprising the step of:

(g) exposing said isolated fusion protein to conditions effective in digesting said fusion protein so as to release said protein of interest from said fusion protein, thereby obtaining a released protein of interest.

7. The process of claim 6, wherein said conditions effective in digesting said fusion protein so as to release said protein of interest therefrom are selected from the group consisting of proteolysis effected via a protease and proteolysis effected under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

9. The process of claim 2, further comprising the step of:

(e) exposing said fusion protein cellulosic matter complex to conditions effective in digesting said fusion protein so as to release said protein of interest therefrom, thereby obtaining a released protein of interest.

10. The process of claim 9, wherein said conditions effective in digesting said fusion protein so as to release said protein of interest therefrom are selected from the group consisting of proteolysis effected via a protease and proteolysis effected under predetermined cis or trans conditions for digesting a controllable intervening protein sequence.

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